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COMMONWEALTH OF AUSTRALIA

Council for Scientific and Industrial Research

BULLETIN No. 75

Nigrospora Musae n.sp. and its Connexion with "Squirter" Disease in Bananas

By

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FOREWORD.

For several years past, the Council has interested itself in the development of better methods for the ripening and transport of Australian bananas from the producing areas of the north to the important markets in the south.

In these markets, in particular, the so-called "squirter disease" is one of the troubles with which the industry is confronted. Consequently, when the Botany Department of the University of Melbourne indicated that it would investigate the problem, the Council heartily welcomed the decision.

The investigational work has now reached a stage where the results warrant publication, and they are set out in the pages that follow.

In making this work generally available, the Council tenders its very cordial thanks to the University, particularly to the Botany Department, and to the investigators concerned for the work they have done and for their kind acquiescence in the suggestion that their results might be published as one of the Council's Bulletins.

SUMMARY.

The symptoms, distribution of "squirter" in the field, and previous work on this disease are briefly discussed.

A fungus which was repeatedly isolated from the rotting tissue and also from flecked banana leaves has been identified as a new species of *Nigrospora,* and described under the name *N. Musae* n.sp. Its appearance in the fruit tissues and on culture media is described.

The temperature relations of this fungus have been investigated, and it has been found that the optimum temperature for growth is in the region of 19° C., and that growth ceases at temperatures above 32° C. The highest percentage of spore germination in banana juice was obtained between 19–22° C. The temperature limits for growth of *N. Musae,* as well as cultural characters, separate it from other members of the genus.

Nigrospora canescens n.sp. was also isolated from flecked banana leaves. This latter species is, however, non-pathogenic to the green banana fruit.

The presence of hyphae in the vascular tissue of the banana stalk has been demonstrated, and the path of infection into and through the fruit traced. The characteristic half inch or so of unaffected flesh at the stalk end of the fruit in young "squirter" has been shown to be the result of the restriction of the pathogen, during the early stages of the disease, to the vascular tissue until it reaches the ovular region.

Inoculation experiments have shown that *N. Musae* is pathogenic to the injured fruit, and if the inoculum is placed into or on the cut ends of the stalks of green bananas, "squirter" symptoms can be artificially produced in the laboratory, provided favorable conditions of temperature and humidity are maintained during the course of the experiments.

The seasonal nature of "squirter" has been associated with the temperature range of the fungus. It is during the winter and early spring that plantation and transport temperatures are favorable to the growth of *N. Musae,* and render infection on the field and subsequent growth of the pathogen during rail transport both possible and probable.

The localization of "squirter" to fruit ripened in the southern markets has been correlated with the method of packing fruit in "singles," a method until recently not adopted in Brisbane, where "squirter" was formerly practically unknown.

Nigrospora Musae n.sp. and Its Connexion with "Squirter" in Bananas.*

I. INTRODUCTION.

The banana industry in Australia is concerned chiefly with the Cavendish variety (*Musa Cavendishii*), and the actual area from which the commercial fruit is obtained is confined to the more southern coastal parts of Queensland and northern New South Wales, an area approximately bounded by latitudes 26° 15′ S. and 29° 40′ S. The fruit for the southern markets of the Commonwealth has, therefore, to be transported long distances by rail, later ripened, and passed into the retail market. A disease known as "squirter" has for a number of years caused serious damage to such bananas in both Sydney and Melbourne.

1. Symptoms.

Squirter is characterized by a discolouration and soft rot of the core, the flesh becomes brown and mealy-looking, and in the late stages breaks down to a liquid mass. When any pressure is applied to the fruit, the liquid will squirt out, and so the vernacular name "squirter" has been generally given to this condition. In the early stages of the disease, a very characteristic feature is the presence of a zone of perfectly healthy flesh immediately underneath the stalk of the fruit, and which extends inwards for about half an inch, and separates the discoloured and rotting core from the actual stalk of the fruit (Plate 1, Fig. 1). As squirter develops, this symptom disappears, the rot extends back to the stalk region, and the discolouration and liquefaction of the entire flesh at the stalk-end results; the rot then rapidly extends right through the flesh of the fruit. As Goddard states(7): "It is impossible to detect any signs of 'squirter' potentialities in green bananas before their entry to the ripening rooms. . . . The earliest indication of definite squirter is occasionally recognized during the colouring of the outer skin of the fruit from green to faint yellow. This is discernible in the ripening room, becoming more pronounced about 24 hours after the fruit has been released from this room, and increasing in intensity each succeeding day. A very high percentage of the squirter fruit becomes a mass of decayed pulp within four to eight days after release from the ripening rooms. Thus the retail seller and the consumer are the main sufferers."

2. Distribution of "Squirter" in the Field.

Until very recently, squirter has been troublesome only in bananas separated from the hands, and packed as "singles" in cases on the plantation, for transport to Sydney and Melbourne—distances of about 600 miles and 1,200 miles respectively. It was for a long time practically unknown in Brisbane. The disease, however, had been found in the latter city as it occasionally developed in samples of cased

* Typescript received for publication 15th May, 1933.

bananas used for experimental purposes, and during the latter part of 1932, appeared in commercial fruit. The definite appearance of the disease in the commercial sphere synchronized with a change in the method of handling bananas for the Brisbane markets. Up to 1932, the fruit had been ripened in bunches, but it has recently been thought desirable to change this procedure, and it is now marketed in Brisbane chiefly as "singles" in cases as for the southern trade. Later, an attempt is made in this report to explain the apparent immunity from squirter which was so marked in Brisbane fruit under the old method of marketing, and its former localization to the southern markets of the Commonwealth.

It is difficult to trace, with any great certainty, those plantations which have produced squirter bananas, so that the actual distribution in the field of potential squirter fruit is not accurately known. The chief commercial plantations, however, lie south of Gympie in Queensland, and extend as far south in New South Wales as Coff's Harbour. Squirter fruit has been recorded from many plantations throughout this region and also from Norfolk Island. The trouble appears to be especially prevalent in the neighbourhood of Murwillumbah in New South Wales, and Brooloo and Woodford in Queensland. Only rare and isolated examples of squirter fruit are known from plantations north of Gympie, but towards the end of 1932, one case of bananas from Sarina (near Mackay, Queensland, 21° S.) received in Brisbane gave 30 per cent. squirter. The disease shows a well-marked seasonal rhythm, is recognized as a trouble of winter and early spring fruit, and is not troublesome during the warmer periods of the year.

3. Previous Work.

In 1925, an investigation of squirter in bananas was undertaken by Goddard, who later published a preliminary report on the subject(7). He considered that the condition was a physiological one, and devised and suggested experiments to test out this hypothesis.

In an article on banana diseases in Fiji, Campbell(5) reported a softening and watering of the pulp of apparently green fruit—a condition which he could not explain on the meagre information then available, but suggested that in this connexion it was of interest to note that a disease—squirter—was troublesome in Queensland bananas after they had been in transit for some time.

As a result of Goddard's work, the theory most favoured to explain the cause of squirter was that the physiological breakdown probably developed during the transport of the fruit by rail through the highlands of New South Wales where adequate protection from sudden changes of temperature was not assured. Young, Bagster, &c. (24), in a bulletin dealing with ripening and transport of bananas in Australia, briefly refer to certain diseases, including squirter, which develop during these periods. Although the experiments carried out by them were not undertaken primarily from the squirter point of view, they nevertheless give some data to show that there is less tendency for squirter to develop after transport in fruit packed in full hands, than in fruit packed in "singles"; and also if fruit is cased as "singles"

and kept in Brisbane for some days at an average temperature of 50–70° F. before ripening, then squirter appears to the same extent as in a similar sample sent to Melbourne and ripened in the ordinary way. They also found that after storage at low temperatures, only certain lots of fruit gave a high percentage of squirter. "Material from some plantations appears to be always free from squirter, whilst apparently similar fruit from other plantations, carried and ripened under almost identical conditions, frequently varies in squirter development from none at all to 20 per cent. or more." They considered that the ripening experiments suggested that the trouble was due "in part to some pre-disposing condition developed in the plantation."

King(9), also assuming the physiological character of squirter, set out to test the theory which had at the time been formulated "that the primary cause was related to climatic or other conditions on the plantation." He concentrated on an investigation of possible soil factors which might be operating in the production of squirter. His results demonstrated, however, that squirter occurs on all types of soil ranging from poor soil which had grown bananas for twelve years without fertilizer, to rich new land in its first cropping year.

The investigation into the cause of squirter described in this present paper was commenced last year at the suggestion of Associate-Professor W. J. Young, who collaborated with the authors in undertaking the ripening of experimental bananas under conditions comparable to those employed commercially. Subsequently, it came to our knowledge that in an unpublished note to Professor Bagster dated 4th October, 1931, Dr. Herbert, of the Queensland University, stated that he had found septate fungal hyphae in some squirter bananas examined by him, and that on transferring portion of the diseased tissue to healthy bananas these developed a rot suggestive of squirter. Fungal hyphae commonly develop in diseased tissue in plants as a result of secondary invasion, and as the fungus was undetermined no certainty could be attached to Herbert's observations. In a letter to one of the authors in July, 1932, Herbert states that he had not identified the fungus stated to have produced the rot in the previous year. The later published investigations carried out by others in Queensland ((24, pp. 48-49 and 52), (9)) were continued on the assumption that the disease was a physiological one and not due to a pathogenic organism.

As it was early established in our work that a particular fungus was constantly present in squirter bananas, the investigation developed along two main lines—

(1) The organism isolated from squirter tissue was studied in detail from a morphological, physiological, and systematic view-point.

(2) Inoculation experiments were carried out, using various methods of inoculation, and varying the conditions of the experiments to determine the pathogenicity of this organism and some related forms, to the banana fruit.

II. THE FUNGUS ASSOCIATED WITH SQUIRTER TISSUE AND ITS SYSTEMATIC POSITION.

1. Its Characters in the Fruit and on Culture Media.

The mycelium in the flesh of the banana ramifies through and between the cells, and appears as coarse, hyaline, septated hyphae with dense protoplasmic contents, which vary in diameter, ranging approximately from 3-10μ. Only the vegetative condition occurs in the banana, and no sporing stage has ever been observed by us in the diseased tissue. When isolated from the flesh and cultured on malt agar, the fungus is sparse in its growth, although it spreads rapidly in a horizontal direction. It appears at first as a white, water-soaked, more or less etched colony, with very characteristic cottony white strands growing down from the inoculum towards the lid of the plate. As the colony spreads across the plate, these strands grow copiously, and the whole surface becomes covered with them and presents an appearance like cotton wool. The upper surface remains quite white, but the medium on the lower surface, after a few days, becomes an olive-citrine colour [Ridgway(15)] which later intensifies until it is almost black; the submerged hyphae are also very dark in colour. In culture, the fungus remains in the vegetative condition for some considerable time, but eventually spores appear scattered sparsely through the colony. The length of time which elapses before the sporing stage commences and the density of sporing vary somewhat with the different isolations. The spores are light brown at first, but soon become intensely black. They are sub-spherical, round in surface view but oval when seen from the side, borne terminally on short inflated conidiophores or at the ends of side branches from the main hyphae as is typical for the genus *Nigrospora* Zimmermann, and measure on an average 15 x 18μ.

2. The Described Species of the Genus *Nigrospora*.

Mason(9), after a considerable discussion of many of the black-spored Hyphomycetes which have been recorded on Monocotyledons, referred them to the genus *Nigrospora*, and listed four species on these plants, viz.:—*N. Panici* Zimm., *N. Oryzae* (B. and Br.) Petch, *N. sphaerica* (Sacc.) Mason, and *N. Sacchari* (Speg.) Mason. Jaczewski(8) has since recorded *N. Gossypii* which he found on immature cotton bolls in Russia.

N. Panici was found by Zimmermann on leaves of *Panicum amphibium* in Java, and was made the type species of the genus. He did not succeed in getting it into culture, but records size of spores obtained from the host as 20-25 x 25-30μ.

In 1913, *N. Oryzae* was recorded as occurring on the leaves of the banana in West Indies under the name *Acremoniella occulta* Cav.(1). Later, Tomkins(18), who studied the wastage of bananas in transport from Brazil to Europe, included *N. Oryzae* in an incidental list of fungi which he isolated from Cavendish bananas, but the fungus apparently caused no trouble under the transport conditions which exist between these two countries. Wardlaw and McGuire (21.22.23) simply refer to the genus in their reports on investigations of diseases of the banana in storage and transport; while Beeze(2.3)

states that he has not found *N. Oryzae* on bananas arriving in Europe (Germany) from the West Indies, Brazil, and the Canary Islands. This species has been known in mycological literature under several names. It was first described by Berkeley and Broome(4) as *Monotospora Oryzae.* Petch(13) re-described *M. Oryzae* B. and Br. as it occurs on maize, and referred it to Zimmermann's genus *Nigrospora* as *N. Oryzae* (B. and Br.) Petch. Prior to this, however, an isolation from *Phragmites* was described and figured by Molliard(12) as a new genus and a new species under the name *Basisporium gallarum.* The fungus was described from cultures, and as pointed out by Mason the description shows clearly that *B. gallarum* Moll. is a *Nigrospora* with spore dimensions fitting those of *N. Oryzae.*

N. sphaerica (Sacc.) Mason has also been recorded from the banana, being isolated from the skin of the fruit and the petioles(10). In its appearance on the host, it is very similar to *N. Oryzae,* but it is separated from the latter species on account of its larger spores.

N. Sacchari (Speg.) Mason has been reported on sugar cane, and although not known in culture, spore measurements taken from the host set it apart as a very large-spored form.

N. Gossypii Jacz. has been isolated from immature cotton bolls in Russia by Jaczewksi(8). The fungus resembles *N. Oryzae* morphologically, and has spores measuring 12 to 13.6μ in diameter, but since *N. Oryzae* has only been reported on Monocotyledons, and not as yet on those in Russia, Jaczewski has created a new species for his isolation.

3. A Comparative Account of the *Nigrospora* Isolated from "Squirter" Fruit and Some Allied Forms.

The two last-named species (*N. Sacchari* and *N. Gossypii*) have not been considered in attempting to determine the systematic position of the Australian isolation. The very large spores of the former and the incomplete description of the latter places them beyond our field of comparison. The remaining species of the genus (*N. Panici, N. Oryzae,* and *N. sphaerica*) were obtained in culture from the Centraalbureau voor Schimmelcultures Baarn, Holland. According to Mason, they are primarily distinguished by spore size. *N. Oryzae* is a small-spored form with the long diameter of the spores averaging from 14 to 16μ, while in *N. sphaerica* the long diameter averages from 16 to 18μ. These mean spore sizes show no tendency to run into one another, and are, moreover, accompanied by distinctive cultural characteristics.

Zimmermann's measurements for *N. Panici* obtained from spores from the host plant were very large being 22-25 x 25-30μ. A culture obtained by us from Baarn as *N. Panici* isolated by Kuyper from an unstated host possesses smaller spores. Our measurements of the spores obtained from this material as well as those made by Mason who handled a similar culture, are considerably less than those given by Zimmermann, as they averaged 17 to 19μ, approximating, therefore, in spore size to *N. sphaerica.* Whether this culture really represents Zimmermann's species is not quite certain; it is, however, very distinct from *N. sphaerica* in its cultural characteristics, and so we are content to retain it apart from *N. sphaerica* as *N. Panici* Zimm.?

As the spore measurements of the *Nigrospora* sp. isolated from squirter tissue average 15 x 18μ, this fungus corresponds in spore size with both *N. sphaerica* and our measurements of *N. Panici*? Accordingly, it is with these last-named species that a further comparison must be made. The growth of all three species was compared on a number of different media, but the squirter isolation appeared quite distinct on all. A description of the appearance of a ten-day old culture at room temperature on malt agar in a petri dish of each of the three forms is given in Table I.

TABLE I.—COMPARISON OF SPECIES OF NIGROSPORA.

N. sphaerica (Sacc.) Mason.	*N. Panici* Zimm. ?	*Nigrospora* sp. from squirter tissue.
Upper surface floccose to woolly. A very few columnar-like hyphal strands reach to the lid but do not obscure the surface of the colony which appears dark owing to colour developed in the medium. Lower surface olivaceous black in centre, sepia on edge.* At end of four days, colony 5 cms. in diameter.	Upper surface felted to powdery. No hyphal strands are formed. Faint tinge of cream to pink in centre, the darker colour of the medium occasionally showing through the surface. Lower surface capucine orange to faint olive citrine towards centre.* Colony 8 cms. in diameter at end of four days.	Upper surface white and like cotton wool in appearance. Hyphal strands very numerous, reaching to lid, much branched at base. The copious development of these strands obscures the upper surface of the medium. Lower surface uniformly olive citrine.* Colony 8 cms. in diameter at end of four days.

* Ridgway (15).

The rate of growth at different temperatures also marks the *Nigrospora* from squirter tissue as distinct from either *N. Panici* or *N. sphaerica*. To find the effect of temperature on the rate of growth, plates of malt agar were inoculated at the centre with discs of agar cut from a plate culture of the species in question. The discs were cut out, as nearly the same size as possible, around the circumference of an imaginary circle drawn midway between the centre and edge of the dish. Three plates were incubated for each species at each temperature. The diameter of each colony was measured daily, and the rate of lateral spread so determined. Fig. 1 represents the results obtained using the *Nigrospora* from squirter as the source of the inocula.

At 6.5° C., growth was very slow, the colony measuring only 1.6 cms. in diameter at the end of six days. At 32° C., there was practically no growth; a few mycelial strands were put out, but growth did not reach 1 cm. in diameter at the end of a week. The optimum temperature was 19° C., when the fungus grew rapidly and sent strands of mycelium down to the lid of the plate. At 25-28° C., the growth was much slower and thicker, the entire surface being covered by a thick weft of mycelium which reached down to the lid. The lower submerged hyphae darkened the medium more quickly and more intensely at these higher temperatures. The maximum temperature at which growth takes place is 32.5° C. Figs. 2 and 3 represent the results obtained when *N. Panici* and *N. sphaerica* respectively were used as the inocula.

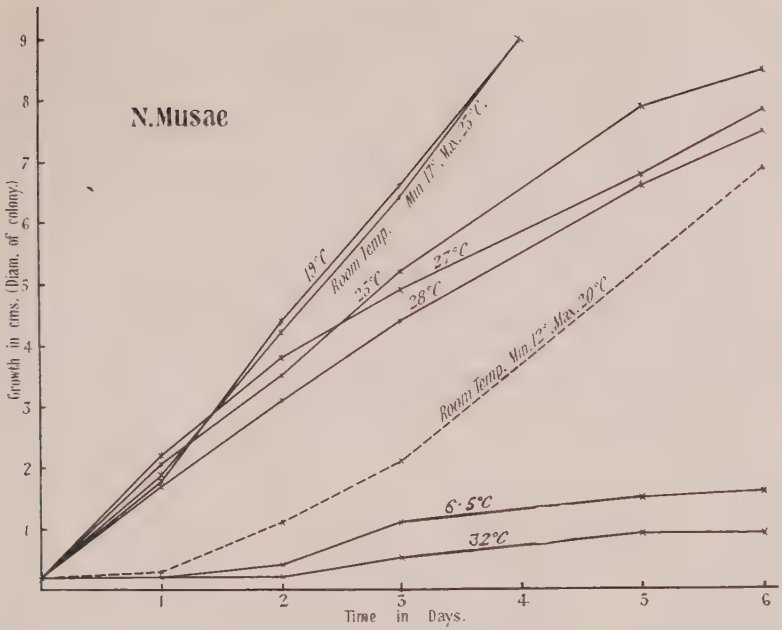

FIG. 1. Growth of *N. Musae* on malt agar at different temperatures.

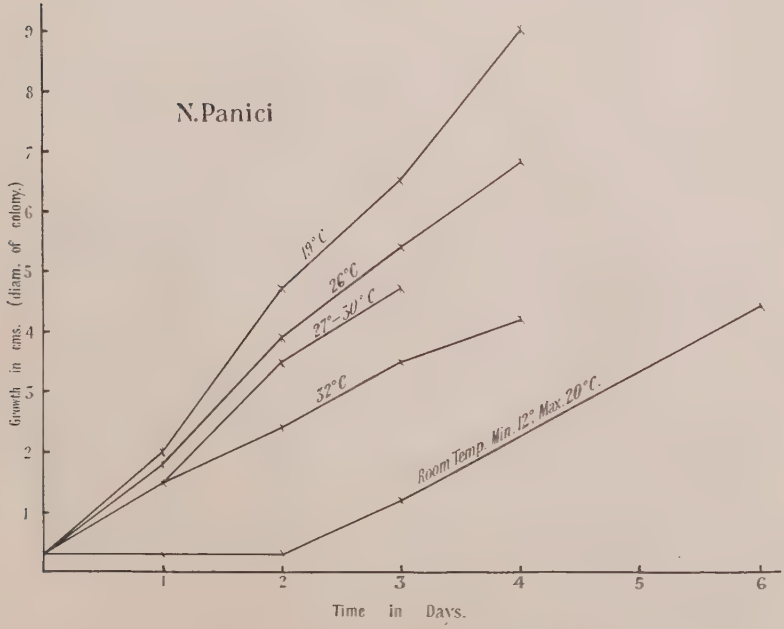

FIG. 2. Growth of *N. Panici* on malt agar at different temperatures.

It will be seen that the optimum temperature for *N. Panici* lies in the region of 19° C. At this temperature, as well as at 26° and 27°, the rate of growth approximates very closely to that shown by *N. Musae*. At 32° C., however, a marked difference is noticeable; the former still grows vigorously, whereas the growth of the latter is practically inhibited. The maximum temperature for growth of *N. Panici* lies between 32° and 37° C., for at the latter temperature no growth occurred. At these higher temperatures, sporing became more abundant, with the result that the upper surface became darker in colour.

N. sphaerica has a much slower linear rate of growth than either *N. Musae* or *N. Panici*, and there is little difference in the diameter of the colonies when grown at 19°, 26°, and 27-30° C. The optimum temperature is again about 19° C., and the maximum at which growth occurs about 30° C. There is no growth at 32° C., so that in this respect *N. sphaerica* resembles *N. Musae*.

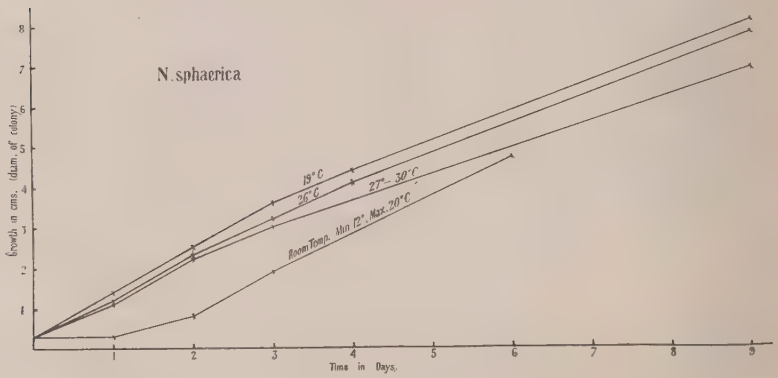

Fig. 3. Growth of *N. sphaerica* on malt agar at different temperatures.

N. Oryzae (as well as *N. sphaerica*) has been recorded from the banana in the Northern Hemisphere, but, as stated previously, the spore measurements of this species are smaller than those of the squirter organism. Its cultural characters, however, tend to resemble the latter fungus, so its growth rates at different temperatures were also determined for comparative purposes. (See Fig. 4.)

The optimum temperature for growth in this species lies between 27-30° C., and at these temperatures linear spread is rapid. At 19° C.—the optimum temperature for the squirter fungus—growth is slower. At all the temperatures, rapid and copious sporing took place in strong contrast to the behaviour of the squirter organism, which in culture media spores sparsely and only very slowly at any temperature.

As the fungus isolated from squirter bananas differs both in its cultural characters and in its temperature relations from those described species of the genus with which it has been compared, it appears to be an undescribed species of *Nigrospora* for which we suggest the name *Nigrospora Musae* n.sp. This is the first record of the genus for Australia.

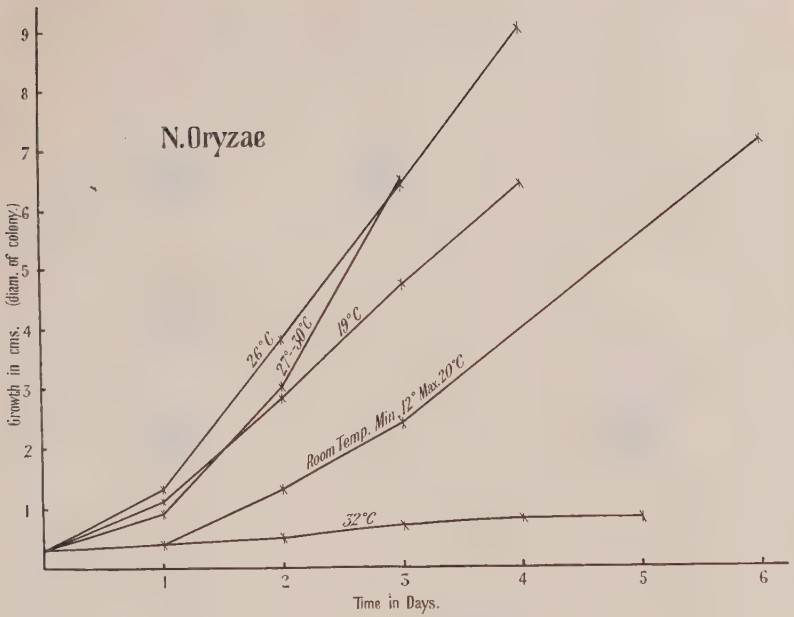

FIG. 4. Growth of *N. Oryzae* on malt agar at various temperatures.

4. Specific Description of *Nigrospora Musae*.

Nigrospora Musae n.sp.

Hyphae at first hyaline, the submerged ones quickly turn brown, they are much septated, and contain a large amount of oil. In culture, the aerial mycelium remains white, is copiously developed, and cotton-wool-like strands pass to the lid of the dish; the lower surface of the medium is coloured olive-citrine. Spores are formed sparsely, and occur on the slightly inflated tips of a more or less branched conidiophore. (See Fig. 5.) When mature, they are jet-black and often surrounded by a coat of mucous. They are sub-spherical, 18μ in their long diameter (across the poles), and 15μ in their short diameter (in the axis of the poles).

Minimum temperature for growth on malt agar	5° C.
Optimum temperature for growth on malt agar ..	19° C.
Maximum temperature for growth on malt agar..	32.5° C.

Pathogenic to the injured green banana fruit.

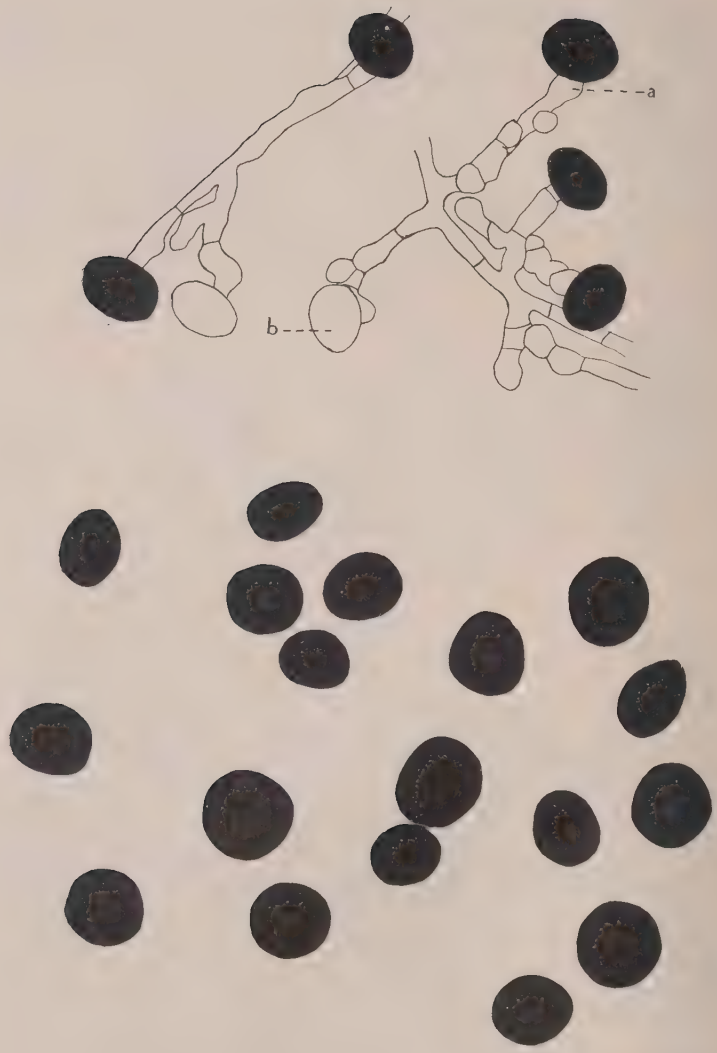

FIG. 5. Conidiophores and spores of *N. Musae*—(*a*) inflated tip of conidiophore, (*b*) young unpigmented spore. × 400

III. ISOLATIONS FROM BANANA LEAVES.

1. *N. Musae* as well as another undescribed Species of *Nigrospora* isolated from Banana Leaves.

N. Musae was isolated on several occasions from flecked banana leaves which were sent to Melbourne from Brisbane for examination. Although the leaves showed numerous small flecks which were varied in type, *Nigrospora* conidiophores and spores were never seen *in situ* on the leaf, although the surface was examined carefully with a hand lens, and many hand sections were made through the browned or blackened areas.

In addition to *N. Musae,* another *Nigrospora* was isolated from the leaves; unlike the former, it was never obtained from the fruit. On culture media, it spored more quickly and more abundantly than *N. Musae,* and on the average the spores were a little larger (17 x 19μ) than those of *N. Musae, N. Panici,* or *N. sphaerica.* It is readily separated from them by its distinct cultural characters.

On malt agar, a ten-day old culture showed a cottony upper surface due to vertical strands of hyphae, which, however, did not grow to the lid of the dish as in *N. Musae.* The colony, too, was distinctly zoned; the zones were due to rings of thicker, floccose, mycelial strands which alternated with rings of shorter, thinner strands. The whole was a dirty cream colour. The mycelium continued to grow even after the edge of the dish was reached, and the sides became covered with mycelium. The lower surface was pale orange to orange buff in colour.

This form has not such a restricted range of temperature as *N. Musae,* for although it did not grow at 5° C., it grew quite well at 32° C. The growth rate dropped off quickly above this temperature, and there was no growth at 37° C. The results of growth at different temperatures are shown in Fig. 6.

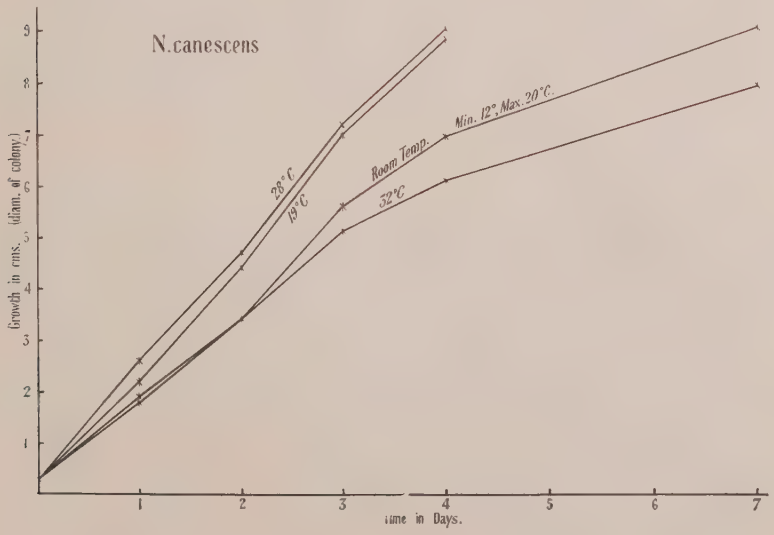

FIG. 6. Growth of *N. canescens* on malt agar at various temperatures.

It will be seen that the optimum temperature for growth is 28° C., although good growth occurs over a wide range of temperature, even at 19° C. At this latter temperature, the upper mycelium was whiter than at the high temperatures, and did not show zoning.

It is difficult to describe the differences in the types of growth shown by all these forms in culture, but the differences are constant, and one can always readily identify the forms appearing on the plates. This fungus does not agree with any of the previously described species of *Nigrospora*, and as it is quite distinct from *N. Musae*, we suggest the name *Nigrospora canescens* n.sp.

2. Specific Description of *Nigrospora canescens*.

Nigrospora canescens n.sp.

Hyphae hyaline or faintly tinged with pink, some later becoming dark brown, much septated. Short floccose aerial mycelium is copiously developed; on malt agar, the lower surface of the medium is coloured pale orange to orange buff. Spores are formed abundantly, and are borne on the inflated tips of a much branched conidiophore. When mature, they are jet-black and sub-spherical, 19μ in their long diameter, and 17μ in their short diameter.

No growth on malt agar at 6.5° C.

Optimum temperature for growth on malt agar—28° C.

Maximum temperature for growth on malt agar—above 32° C.

Not pathogenic to the injured green banana.

IV. GERMINATION OF SPORES OF NIGROSPORA MUSAE.

Nigrospora Musae does not form spores very readily, and it was difficult to obtain sufficient to carry out satisfactory germination tests. Preliminary experiments showed successful germination in drop cultures of sterilized banana juice, but practically no result was obtained in distilled water.

It was noticed by Ramsey(14) that spores of *Basisporium gallarum* Moll. = *Nigrospora Oryzae* (B. and Br.) Petch germinated readily in tomato juice, but that practically no spores germinated in distilled water. When, however, drop cultures with distilled water were placed in chambers containing whole tomatoes or tomato pulp, high percentage germination resulted. Durrell(6) in his work on the germination of spores of *Basisporium gallarum* Moll. showed this result to be due to the carbon dioxide respired from the fruit. Durrell obtained a high percentage of germination in distilled water when the drop cultures were placed in chambers with different kinds of fruit peel, including banana peel, various flowers, and whole fruits. In all cases, he showed it to be due to the carbon dioxide evolved by these parts. With distilled water alone, germination took place after several days, and he suggested that by this time enough carbon dioxide had been absorbed to stimulate germination. Similar experiments were carried out by

us with spores of *N. Musae* in distilled water exposed on slides on moist filter paper in large petri dishes containing banana peel, but in no case was the percentage of germination higher than with distilled water alone. The scarcity of spores, however, did not enable us to repeat these experiments, so the result is not absolutely conclusive.

Savulescu and Rayss(16) carried out extensive experiments on the germination of *Nigrospora Oryzae* (B. and Br.) Petch from maize. They found that after one and a half years the spores from diseased maize cobs had completely lost their germinative capacity, and that the percentage of germination in maize-meal extract varied with the temperature, 100 per cent. being obtained after 24 hours at temperatures between 20–30° C. Germination tests were made for *N. Musae* in banana juice and distilled water at different temperatures, and it was found that the optimum temperature after 24 hours lies between 19–22° C. The results are shown in Table II., and are expressed graphically in Fig. 8. The appearance of the spores germinating in banana juice is indicated in Figs. 7, 9, and 10.

TABLE II.—PERCENTAGE GERMINATION OF SPORES OF N. MUSAE AT VARIOUS TEMPERATURES.

	Temperature°C.						
	6.5.	19.	Room 15–25.	25.	28.	32.	37.
Percentage germination in distilled water after 24 hours	0	9·5	38	7	1·5	1	0
Percentage germination in banana juice after 24 hours	2	65·5	68	30	31	27	0

Although the highest percentage germination, after 24 hours incubation, is found at 19–22° C., germination commences more rapidly in banana juice at higher temperatures, e.g., after six hours at 32° C., 20 per cent. had germinated, while at 19° C. very few germinating spores could be found. It is interesting to note that, although the mycelium of *N. Musae* does not grow at 32° C., nevertheless 27 per cent. spore germination was obtained in banana juice at that temperature. The germ tubes formed at 32° C. are short, wide, unbranched, and remain restricted in length. (See Figs. 9B and 10.)

At 25° C. and 28° C., the germ tubes branch copiously (Fig. 9A). In distilled water at these temperatures, where germination does take place to a small degree after 24 hours, the germ tubes are narrow and sparsely branched.

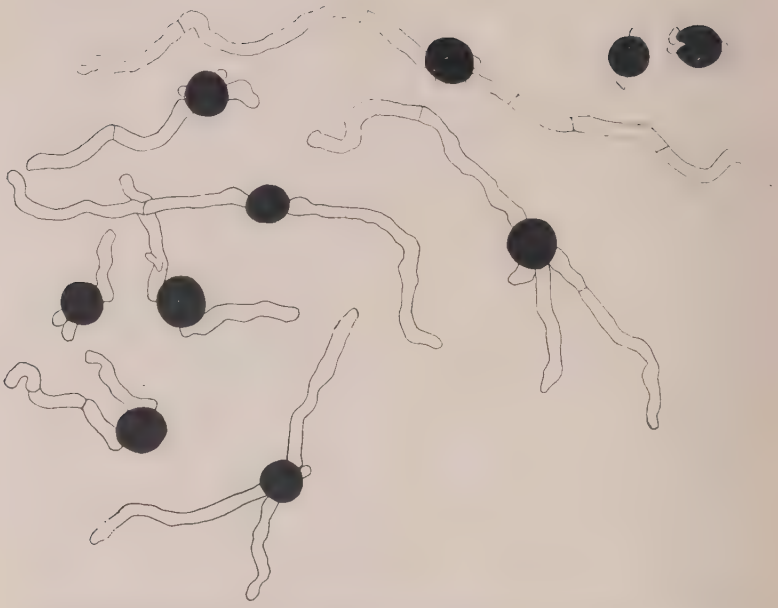

FIG. 7. Typical appearance of germinating spores of *Nigrospora Musae* in banana juice. × 250.

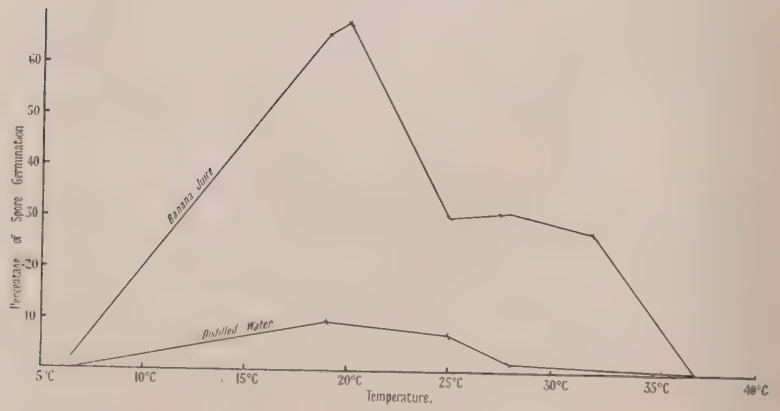

FIG. 8. Influence of temperature on the germination of spores of *N. Musae*.

FIG. 9. Germination of spores of *N. Musae* after 24 hours in banana juice A, at 27°C. (note branching); B, at 32°C., short, wide unbranched germ tube. × 100.

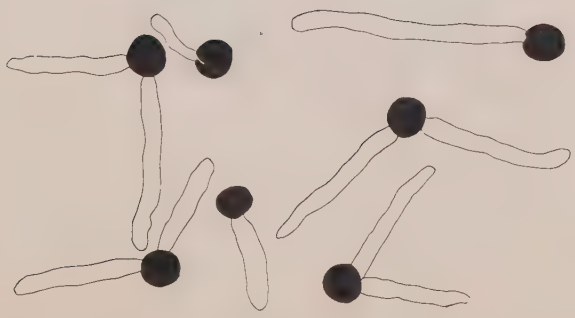

FIG. 10. Germination of spores of *N. Musae* in banana juice after 24 hours at 32°C. × 260.

V. DISTRIBUTION OF HYPHAE IN SQUIRTER BANANAS.

When an infected banana in an early stage of the disease is cut vertically, the unaffected half inch of flesh next to the stalk, in contrast to the more deeply-seated discoloured tissue of the core, appears as a most characteristic and constant symptom. At first sight, this phenomenon appeared somewhat puzzling, but as it became evident that a pathogen was responsible for the squirter condition, it was assumed that entry was probably gained at the cut or torn end of the stalk, and a histological examination of the fruit was undertaken to seek for an explanation of this healthy zone of pulp tissue which seperates the diseased area of the fruit from the stalk in the early stages of the trouble. Longitudinal sections of the stalk of a squirter fruit have always shown one or two, but never many, hyphae passing down the vessels of the xylem tissue. (Fig. 11.)

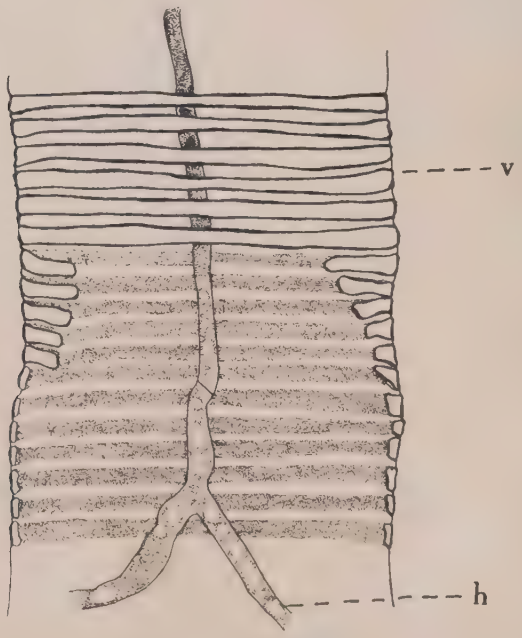

Fig. 11. Vessel from the stalk of a "squirter" banana showing the presence of a fungal hypha. v = wood vessel. h = hypha. × 625.

The scarcity of hyphae is no doubt due to the fact that infection of the fruit is accomplished by a spore which, lodged in the broken end of the stalk, germinates in the sap that exudes from the cut surface. The germ tube forces its way down a xylem vessel in the stalk, branching but little in this fibrous tissue, and eventually reaches one

of the vascular strands which supplies the carpellary tissue. It is often difficult to demonstrate the existence of such hyphae in the stalk of squirter fruit, for, if only one spore is responsible for infection, careful search must necessarily be made for the vessel or vessels through which the organism has gained entry into the pulp. However, we have never failed to find the presence of a few hyphae in the stalks of any squirter bananas examined by us. There are three main vascular bundles passing down from the stalk into the central carpellary core, and it is not until the first abortive ovules are reached that lateral ovular strands are given off. The fungus, which up to this point remains confined to the vascular tissue, now passes into the flesh. An examination of transverse sections of the banana fruit reveals the path by which the fungus can pass into the flesh at this point.

Fig. 12 shows the three fleshy carpels with their much infolded edges (*e*) obliterating the cavities of the loculi. Vascular strands (*c*) run right around each carpel and pass to the abortive ovules (*a*), but the main supply to the latter is by branches (*b*) from the three central vascular strands (*d*).

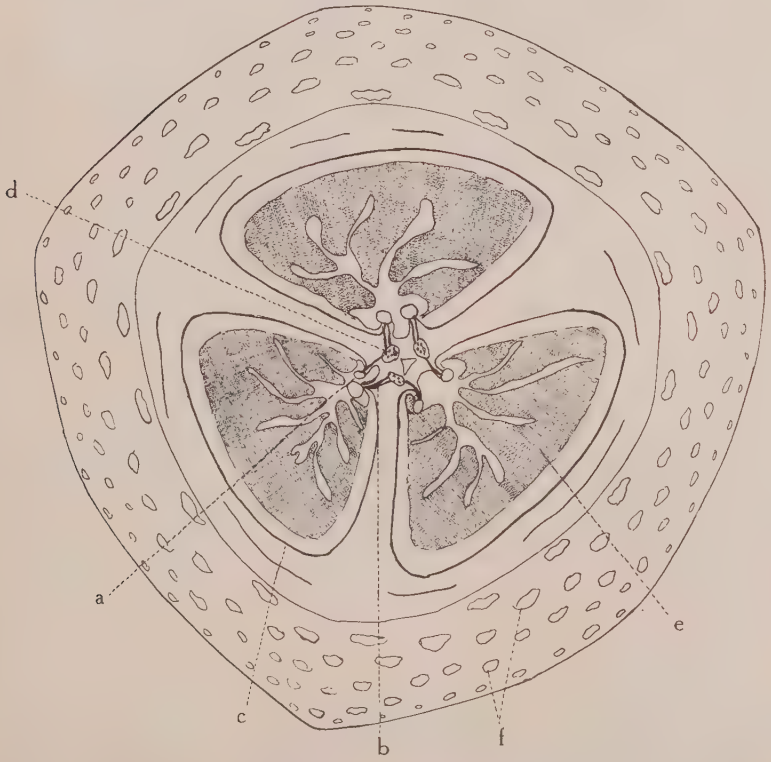

Fig. 12. A diagrammatic transverse section through the banana fruit—(a) ovule (abortive), (b, c) xylem vessels passing to ovule, (d) vascular element passing down core of fruit, (e) much-folded carpel—filling up loculus of carpel, (f) vascular bundles in thalamus.

The vascular supply to the ovular region is shown more clearly in Figs. 13 and 14. Many of the small vascular elements from the main central bundles pass out towards the ovule, so that hyphae passing down these can find their way into the carpellary parenchyma. Once here, a plentiful food supply is available, and so the pathogen has a chance

Fig. 13. Vascular supply of the ovule.—T.S. banana fruit—(a) ovule (abortive), (b) xylem vessels from core to ovule, (c) xylem vessels passing to the ovule from the outside of the flesh, (d) xylem vessels in the core, (e) air spaces around vascular tissue. × 47.

to establish itself. The hyphae spread rapidly through and between the parenchyma cells, and rotting quickly ensues. This deep-seated flesh, which is the first attacked, naturally discolours and rots, and it is only in the later stages, when the mycelium has ramified in all directions that the first half-inch or so is attacked and the rot becomes general.

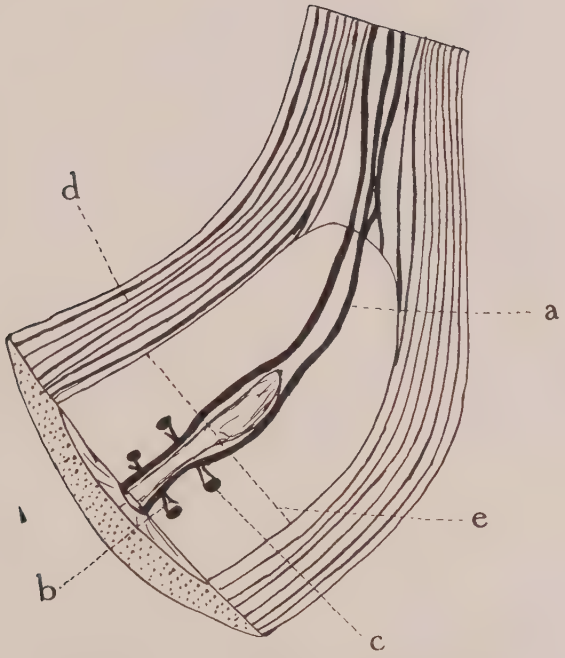

FIG. 14. Diagram of the stalk end of a banana fruit cut vertically showing the course of the vascular bundles from the stalk into the carpels—(a) carpellary bundles, (b) branches to the abortive ovules, (c) abortive ovules, (d) vascular strands in thalamus, (e) line marking off uninfected half inch of young squirter. It is beyond the level of line (e) that rotting first commences, and only later does it extend backwards towards the stalk region.

VI. INOCULATION EXPERIMENTS.

1. Experiments to Test the Pathogenicity of *Nigrospora Musae* to the Banana Fruit.

Parallel with the mycological investigation of the fungus suspected as the organism responsible for squirter, inoculation experiments were planned to test (*a*) its pathogenicity, as well as that of some closely related forms, to the banana fruit, and (*b*) to find the requisite conditions for artificially producing a high percentage of squirter under conditions of storage and ripening.

Several difficulties at once present themselves in such an investigation. If the fungus is placed directly into the flesh of the green banana, by removing, with a sterilized cork borer, a cylinder of skin and flesh and placing a portion of the fungal culture into the cavity, reinserting the plug, and sealing the surface of the scar with melted paraffin, then 100 per cent. rotting is obtained. This method of inoculation undoubtedly proves the pathogenicity of *N. Musae* to the green banana fruit, but although the actual rot is similar to squirter rot in appearance, it does not commence along the core and gradually spread outwards on either side as in natural squirter, nor is the typical unaffected half inch near the stalk end of the fruit evident (Plate 1, Fig. 1). This must be expected, however, when the fungus does not enter through the vascular tissue of the stalk, as is the case under natural conditions. When the fungus is placed directly in the flesh, it spreads on all sides until the whole fruit becomes rotted. The results of experiments carried out in July, 1932, using this method of inoculation, are shown in Table III.

TABLE III.—RESULTS OF INOCULATION OF GREEN BANANAS WITH NIGROSPORA MUSAE.

Temperature.		Total Length of Time in Days.	Inoculated.		Controls.		Remarks.
Before Inoculation.	After Inoculation.		Number Treated.	Number that Developed Squirter Rot.	Number Treated.	Number that Developed Squirter Rot.	
19° C.	26·5° C.	7	7	7	3	0	100 per cent. rot in inoculated fruit 0 per cent. in controls
7·5° C.	26.5° C.	7	12	12	3	0	

Prior to this investigation, it was generally thought that squirter rot of the banana was not due to an organism(7). It was regarded as a physiological disturbance of the fruit, and considerable attention has been given to the effect of various external factors such as fluctuations of plantation temperatures, character of soil, &c., on the incidence of squirter in banana fruit. Unpublished, and some published, data suggested that bananas grown in the cooler parts of the plantation were more liable to develop squirter at ripening than

were those from warmer areas of the same plantation.* For this reason it was thought advisable to determine whether there was any discernible difference in the pathogenicity of *N. Musae* to bananas originating from such "cool" and "warm" spots in the field. The experiments were carried out in the same way as those described for Table III., the results being set out in Table IV. (see also Table IX.).

TABLE IV.—RESULTS OF INOCULATION OF GREEN BANANAS WITH NIGROSPORA MUSAE.

A.—Bananas from "warmer" areas.

B.—Bananas from "cooler" areas.

Expt.	Temperature.		Total Length of Time in Days.	Inoculated.		Controls.		Remarks.
	Before Inoculation.	After Inoculation.		Number Treated.	Number that Developed Squirter Rot.	Number Treated.	Number that Developed Squirter Rot.	
1. A	Room	19° C.	8	12	12	6	0	100 per cent. "squirter" in inoculated
2. A	Room	25·6° C.	8	2	2	2	0	0 per cent. "squirter" in controls
3. B	Room	19° C.	8	13	13	6	0	
4. B	Room	26·5° C.	8	2	2	2	0	

These experiments were carried out on a small scale in the laboratory. The surface of each banana was sterilized, in some cases with alcohol and in others with mercuric chloride, to minimize infection from other sources. After inoculation, the bananas were placed separately in large sterilized jars, and incubated under sterile conditions.

In all the inoculated bananas, the flesh was discoloured, brown and mealy looking, quite soft, and in the later stages of the rot became semi-liquid, just as it does in natural squirter. The controls remained perfectly healthy around the wound, and showed that the injury itself did not contribute to the rotting. The results indicate that *Nigrospora Musae* is definitely pathogenic when inoculated into green bananas. Since it had as much effect on bananas from "warmer" areas as from "cooler" ones, the temperatures to which the bananas have been subjected in the field before picking do not seem to influence the pathogenic properties of this fungus.

Although *Nigrospora Musae* is decidedly pathogenic to green bananas, it had little effect when inoculated into ripe bananas, and caused only a small amount of rotting. Several experiments were done

* Later ripening experiments have shown that there is no real correlation between occurrence of squirter and variation in field temperatures.

with ripe fruit, and in none of them was a typical squirter rot produced. The first method of inoculation used was that described above for green bananas. (See Table V.)

TABLE V.—RESULTS OF INOCULATION OF RIPE BANANAS WITH NIGROSPORA MUSAE.

A.—Inocula on malt agar.

B.—Inocula in banana extract.

Temperature.		Total Length of Time in Days.	Inoculated.		Controls.		Remarks.
Before Inoculation.	After Inoculation.		Number Treated.	Number that Developed Squirter Rot.	Number Treated.	Number that Developed Squirter Rot.	
A Room	21° C.	6	3	0	3	0	Very slight rot in both inoculated and control
B Room	Room	7	15	0	15	0	

In all the early experiments, the *Nigrospora* inoculum was grown on malt agar, but it was later found that if the fungus was grown in liquid banana extract and young cultures used for the inocula, better results followed. Ripe bananas were later inoculated by the latter method and incubated for some days, but again only slight rotting occurred around the injury (Table V.). Such experiments show that *N. Musae* is not so pathogenic to ripe banana flesh as it is to the unripe tissue.

Experiments have shown that the surface of the fruit must be wounded to allow the entrance of the fungus, as it cannot penetrate the intact skin of the banana. Young and old cultures of *N. Musae* on malt agar were placed on the uninjured surface of green bananas and kept moist by binding the area with damp cotton-wool. The treated fruit was placed in humid containers and incubated for some days; however, the fungus was unable to penetrate the uninjured surface. At the same time, a corresponding number of green bananas were wounded by means of two cuts made with a sterile scalpel through the skin to the flesh, and the inocula from young or old cultures of *N. Musae* on malt agar placed on the wounded surface and covered with a gelatine capsule. Rotting resulted in all fruits inoculated with a young culture and to a smaller degree with these inoculated with the old culture, while the wounded controls showed no effect as a result of the actual injury. The rotting in these bananas was not as extensive as usual, for before inoculation the green bananas had been in the ripening rooms with ethylene for 24 hours. The results are shown in Table VI.

Two of the injured control bananas showed slight rotting; from the rotted tissue of one, a *Mucor* sp.? was isolated, while *N. Musae* was isolated from the other.

With the knowledge that *N. Musae* is pathogenic to the wounded banana fruit, we next tried to reproduce the characteristic symptoms

of natural squirter in the laboratory. To do this, it is essential that the fungus should be so placed on the fruit that it gains entrance through the stalk vascular tissue as it does under natural conditions. Many difficulties are encountered when the inocula are introduced in or on the stalks, and it is doubtful whether one will ever be able to produce with certainty more than 50 per cent. typical squirter rot in experiments of this kind. Our work along this line has been somewhat handicapped to date for we have handled green bananas transported south mostly in "singles," and there is necessarily a certain amount

TABLE VI.—RESULTS OF INOCULATION OF INJURED AND UNINJURED GREEN BANANAS WITH NIGROSPORA MUSAE.

A.—Inocula from a young culture on malt agar.

B.—Inocula from an old culture on malt agar.

Method.	Temperature.		Total Length of Time in Days.	Inoculated.		Controls.	
	Before Inoculation.	After Inoculation.		Number Treated.	Number that Developed Squirter Rot.	Number Treated.	Number that Developed Squirter Rot.
A. injured ..	20° C.	Room	8	11	8	11	0
A. uninjured ..	20° C.	Room	8	11	0	11	0
B. injured ..	20° C.	Room	8	11	8	11	2 (slight)
B. uninjured ..	20° C.	Room	8	11	0	11	0

of drying out of the stalk, and also the vessels in the xylem become blocked with gums and tannin, so that the hyphae do not have a free path into the fruit. This difficulty could be surmounted to a great extent by using bananas in "hands" or bunches for such experiments. Drying out of the finger stalks would then to a great extent be prevented or retarded, and so entry through the vascular tissue would be more readily accomplished. Again, in any method of inoculation which has been devised, it has proved difficult to keep the inoculum from drying out before it can establish itself in the stalk tissue. We first cut the stalk vertically for half its length, inserting the malt agar inoculum and binding up the stalk with damp cotton wool. This method was unsuccessful in bulk experiments, for the stalk and the inoculum dried up before the fungus was able to penetrate to the flesh, so that no, or little, rotting resulted. Longitudinal and transverse cuts of the stalk were then tried, the malt agar inocula inserted, and the stalks were finally dipped in melted paraffin. 30 per cent. of squirter rot resulted with this treatment. The low percentage of infection in such an experiment is probably due to the melted paraffin leaking into the cut xylm vessels, blocking them, and so preventing the penetration of the hyphae. The growth of the fungus on the agar is relatively slow, and better results were obtained when the fungus was grown on banana extract in which it spreads more rapidly. The results of the first experiment are seen in Table VII.

TABLE VII.—RESULTS OF INOCULATION OF THE STALKS OF GREEN BANANAS WITH NIGROSPORA MUSAE.

A.—Inocula from a young malt agar culture.

B.—Inocula from an old malt agar culture.

Method.	Temperature.		Total Length of Time in Days.	Inoculated.		Controls.	
	Before Inoculation.	After Inoculation.		Number Treated.	Number that Developed Squirter Rot.	Number Treated.	Number that Developed Squirter Rot.
A. Longitudinal cut	Room	27° C.	19	10	4	5	1
A. Transverse cut	Room	27° C.	19	10	3	5	0
B. Longitudinal cut	Room	27° C.	14	6	0	5	0
B. Transverse cut	Room	27° C.	14	5	1	5	0

More favorable results were obtained when the fungus, grown in sterile banana extract for two to three days, was introduced into the stalk tissue after a core had been removed by a sterile cork borer, the core replaced when the inoculum was in position, and the wound finally sealed with melted paraffin. (Table VIII.).

TABLE VIII.—RESULTS OF INOCULATION OF STALKS OF GREEN BANANAS WITH NIGROSPORA MUSAE.

Inocula obtained from a two-day old culture in banana extract.

Temperature.		Total Length of Time in Days.	Inoculated.		Controls.	
Before Inoculation.	After Inoculation.		Number Treated.	Number that Developed Squirter Rot.	Number Treated.	Number that Developed Squirter Rot.
5° C.	Room	15	22	22	21	0
Room	Room	15	25	25	22	0

A similar method of inoculation was adopted in the next experiment, but as these bananas had been sent to Melbourne as samples of fruit picked from "cool" and "warm" parts of certain plantations, they were kept apart during inoculation and the duration of the experiment. The results again show that the comparatively small fluctuations in temperature to which the fruit are subjected before picking bears no significant relation to the incidence of squirter rot in banana fruit. (Table IX. and also Table IV.)

TABLE IX.—RESULTS OF INOCULATIONS OF GREEN BANANAS RETAINED IN "HANDS" WITH NIGROSPORA MUSAE GROWN IN BANANA EXTRACT (OLD CULTURE).

A.—Bananas from "warm" parts of the plantation.

B.—Bananas from "cool" parts of the plantation.

Temperature.		Total Length of Time in Days.	Inoculated.		Controls.		Remarks.
Before Inoculation.	After Inoculation.		Number Treated.	Number that Developed Squirter Rot.	Number Treated.	Number that Developed Squirter Rot.	
A. Room	Room	16	48	16	51	7	33 per cent. squirter rot in inoculated 14 per cent. in controls
B. Room	Room	16	54	14	56	1	26 per cent. squirter rot in inoculated 2 per cent. in controls

The low percentage of squirter rot obtained in this experiment was probably largely due to the use of an old liquid culture of *N. Musae* as the source of the inocula. In later experiments, good results were obtained when the fungus, grown in banana juice, was placed on the cut end of the stalk and enclosed in a gelatine capsule which fitted over the end of the stalk, and thus served as a damp chamber to keep the fungus from drying out. Under these conditions, growth was rapid, and some hyphae penetrated into the xylem tissue and so into the fruit. Since most of the bananas were in singles, the stalks were not in the best condition for experimental purposes. The optimum conditions for infection were discovered as the summer temperatures became rather too high for active growth of the pathogen, and further experiments of this type will be repeated next season. The results of the experiments using this last method of inoculation are shown in Table X.

During the beginning of November, 1932, when the experiments set on in Table X. were performed, the percentage of "natural squirters" occurring in the bananas was rather high for that time of the year. In experiments 7 and 8 of Table X., the inhibitory effect of low and high temperatures—i.e., 5° and 30° C.—on the action of the fungus is demonstrated. In some of the experiments cited above, the bananas were subjected to different temperatures before and after inoculation, but as long as the temperature after inoculation was favorable to the growth of the fungus, little effect seemed to be noticeable in the final results. In all the experiments, *Nigrospora Musae* was re-isolated from rotting fruits.

TABLE X.—RESULTS OF INOCULATION OF GREEN BANANAS WITH NIGROSPORA MUSAE IN BANANA EXTRACT.

Expt.	Age of Inoculum.	Temperature.		Total Length of Time in Days.	Inoculated.		Controls.		Per Cent. Squirter in Inoculated.	Per Cent. Squirter in Controls.
		Before Inoculum.	After Inoculum.		Number Treated.	Number with Squirter Rot.	Number Treated.	Number with Squirter Rot.		
1	Old culture	Room	Room	14	46	7	24	1 (slight)	15	4
2	Young culture	Room	Room	14	45	42 (5 slight)	24	0	93	0
3	Young culture	5° C.	Room	11	60	33 (7 slight)	94	20	55	22
4	Young culture	Room	Room	10	120	53 (30 slight)	468	51	46	11
5	Young culture	5° C.	Room	9	70	49 (18 slight)	83	26 (18 slight)	70	31
6	Young culture	Room	17-21°C.	11	61	14	68	0	23	0
7	Young culture	Room	29 30°C.	11	64	3 (1 slight)	75	3 (slight)	4·7	4
8	Young culture	Room	5° C.	11	54	8 (4 slight)	65	3	15	4·5

2. Experiments to Test the Pathogenicity of Other Species of *Nigrospora* to the Banana.

The pathogenicities of the other *Nigrospora* spp. which have been recorded from the banana—*N. sphaerica* and *N. Oryzae* for the Northern Hemisphere, and *N. canescens* for Australia—as well as *N. Panici* were tested for comparison with the squirter fungus. (See Table XI.)

TABLE XI.—RESULTS OBTAINED WHEN DIFFERENT SPECIES OF NIGROSPORA WERE INOCULATED INTO GREEN BANANAS.

Inoculum.	Temperature.		Total Length of Time in Days.	Inoculated.		Controls.		Remarks.
	Before Inoculation.	After Inoculation.		Number Treated.	Number that Developed Soft Rot.	Number Treated.	Number that Developed Soft Rot.	
N. sphaerica (culture in banana extract)	Room	Room	8	24	24	24	0	Soft rot in most. *N. sphaerica* re-isolated from rotting tissue
N. Oryzae (culture on malt agar.)	Room	Room	8	24	0	24	0	Not pathogenic
N. canescens (culture in banana extract)	Room	Five days at room temperature then at 25° C.	10	35	0	35	0	Not pathogenic
N. Panici (culture in banana extract)	Room	Room	8	24	0	24	0	Not pathogenic

The bananas which had been inoculated with *N. sphaerica* and subsequently incubated for eight days at room temperature showed a soft brown rot resembling that induced by *N. Musae*, but more restricted in extent. It is interesting to note this result, for *N. Musae* resembles this species (spore size, temperature relations) more closely than any of the other members of the genus.

VII. DISCUSSION.

Since the cause of squirter in bananas had been shown to be due to a fungus, it becomes necessary to discuss several well-known and striking facts concerning the occurrence and distribution of the disease in the light of this knowledge.

1. Squirter is Considered to be Chiefly a Trouble of the Winter and Early Spring Fruit.

Squirter fruit is found in both Sydney and Melbourne during the months of June to October inclusive. This seasonal manifestation offered many difficulties to the varied attempts to correlate the presence of squirter with such factors as soil, conditions of transport, &c., but will admit of a ready explanation when the temperature range for the growth of the pathogen is considered. Growth in culture is slow at temperatures centering around 28° C., and ceases when the temperature exceeds 32° C. The optimum temperature for growth is about 19° C. It is during the winter months that such optimum temperatures for *N. Musae* are registered in the plantations. Records of maximum and minimum temperatures for the months of June and July in several plantations from which squirter fruit had been reported ranged between the temperature limits of 6° C. and 23° C., whereas summer temperatures in the plantations exceed 32° C., so that the growth of the pathogen on the host plant in the plantations would be prevented in the summer months, and therefore spore production would cease and risk of infection of cut stalks considerably reduced. (*N. Musae* was isolated from banana leaves, see page 17.) Even if chance infection occurred, the temperatures during packing and early transport would militate against the establishment of the germ hyphae in the stalk tissue of the fruit, and so squirter would tend to occur only rarely during the summer months. Any unusual spell of cooler weather during this period, however, might result in an outbreak of squirter, and the well-marked seasonal character of the disease which asserts itself under normal conditions would then tend to disappear. It has not been possible to study the actual geographical or seasonal distribution of the fungus in the field, but this phase of the work will be undertaken shortly.

2. Squirter Fruit is Confined in the Main to Plantations South of Gympie (Qld.).

Although it is difficult to obtain accurate information about the geographical distribution of squirter infected fruit, it is a generally accepted fact that squirter is mainly confined to fruit coming from plantations south of 26° 15′ latitude. The banana industry of Queensland is at the present time confined to plantations lying south of

this line. The trouble has also been found in bananas received from Norfolk Island. Early records suggested that squirter had never been reported from plantations lying north of Gympie; however, recent records now include some fruit from Northern Queensland (Sarina, close to Mackay). Until accurate observations have been made in the field, it is not possible to explain the restricted geographical range of plantations from which squirter fruit has been handled. Nevertheless, it is interesting to note that the temperature relations of this fungus may again afford a satisfactory explanation of this phenomenon. Any of the northern plantations which experience winter temperatures falling within the temperature range for growth of *N. Musae* are, however, potential sources of squirter fruit.

3. Squirter is a Trouble of the Southern Markets—Sydney and Melbourne—and until recently has occurred only rarely in Brisbane.

In Brisbane, the fruit has formerly been ripened in bunches, but for various economic reasons it is necessary for the fruit to be transported south in "singles" packed in cases of 200 to 600. Infection occurs before or during packing, and is undoubtedly a result of spore contamination on the cut or torn end of the fruit stalk. If the fruit is kept and ripened in bunches, this danger is never encountered. If the fruit is separated into hands and then ripened, squirter would be definitely restricted in amount, although an occasional fruit might develop the trouble if a spore chanced to lodge on the broken tissue of the stem of the hand. Given ideal conditions, such a spore might establish a mycelium in one or more of the individual fingers, but as the distance the hyphae have to travel, through the vascular tissue of the main stalk as well as through the finger stalk, is so great that in practically all cases the bananas would be transported and ripened before the flesh of the fruit was attacked. This is not the case, however, when the fruit is transported and ripened as "singles," and it is under these latter conditions of transport that *N. Musae* has every opportunity to infect the flesh and so cause the soft rot of the banana soon after the ripening chamber has been reached. In the great majority of cases, however, it is after ripening and during the retailing of the fruit that the heaviest losses by squirter are incurred, for once the fungus is established in the carpellary tissues, ripening of the fruit does not inhibit the spread of the rot.

That infection takes place in the manner just outlined is supported by the fact that microscopic examination of the stalks of squirter fruit always reveals a few hyphae in one or more of the vascular bundles of the stalk. It has been shown that spores germinated well in banana extract at temperatures around 20° C., and only to a smaller degree at 32° C.; also, that at the latter temperature, the germ tubes elongated very little and did not branch. At the present time, it is becoming the practice to transport and ripen bananas in Queensland as singles. Coincident with this change in marketing methods, there was the occurrence of squirter in the Brisbane fruit to a marked degree. This fact is in strict accord with the results of the investigation set out in this report.

4. Fluctuations of Plantation and Transport Temperatures and their Bearing on the Occurrence of Squirter.

Before the pathogenic nature of squirter was established, numerous experiments had been made in an attempt to correlate the condition of such fruit with different temperature conditions prevailing either in the plantations or during transport. The aspects of the various slopes as well as the maximum and minimum temperatures in various parts of a number of plantations were studied and accurately recorded. Fruit picked from those plants growing in the warmer parts were cased separately, and sent to Sydney and Melbourne for ripening at the same time as fruit chosen from the colder parts of the same plantations. The fact that bananas from "warm" areas are as susceptible as those from "cold" areas when inoculated with *N. Musae* in the laboratory indicates that there is no inherent quality in them which makes them resistant to the squirter organism. The percentage of squirter to non-squirter bananas obtained from ripening fruit picked from "warm" and "cold" areas respectively, and transported to the ripening chambers under identical conditions, should not be regarded as significant in correlating the incidence of "squirter" with such temperature conditions, until more is known of the distribution of *N. Musae* in those parts of the plantations from which the fruit has been chosen.

Further work is now in progress in the field in an attempt to locate the main sources of infection and, if possible, to devise suitable control measures for its prevention.

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PLATE I.

Photographs taken by Mr. T. Kilvington.]

FIG. 1 (*top*). Natural "squirter."

FIG. 2 (*bottom*). Banana inoculated with *Nigrospora Musae*.

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